THE DISTRIBUTION OF MINERAL MATERIAL IN THE CALCIFIED CARAPACE AND CLAW SHELL OF THE AMERICAN LOBSTER, HOMARUS AMERICANUS, EVALUATED BY MEANS OF MICROROENTGENOGRAMS [1, 2]

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The histology of the calcified crustacean integument has been investigated by means of the light microscope (Williamson, 1860; Vitzou, 1882; Herrick, 1895; Drach, 1939; Dennell, 1947; Travis, 1955, 1957, 1960) and the polarizing microscope (Prenant, 1927a, 1927b; Dudich, 1931; Drach, 1939). Some aspects of calcification in relation to shell structure have been reviewed by Richards (1951).

The calcium carbonate found in the exoskeleton of the European lobster, *Homarus gammarus*, exists in an amorphous form (Prenant, 1927a). X-ray diffraction patterns obtained from the dry, intact carapace of the American lobster indicate that some calcite is present, but that much of the mineral does not have a distinct crystalline structure (Glimcher, personal communication).

Microroentgenography has been a useful technique in the evaluation of the degree of mineralization in various types of calcified tissue (Engström *et al.*, 1957). This report discusses microroentgenograms of lobster shell which were obtained using comparatively thick sections of calcified integument from the carapace and the claw shell. By exposing the animals to sea water labelled with calcium-45 for a short period of time immediately before sacrifice, labile areas of mineral could be identified.

Materials and Methods

Two male lobsters, 1 (518 grams) and 2 (436 grams), were obtained live from a local market. The lobsters were in intermolt condition, stage C-4 (Drach, 1939). The animals were allowed to remain in a tank of artificial sea water for seven days. They were then placed for two hours in an artificial sea water solution labelled with calcium-45 and strontium-85 which contained 25 mEq./l. calcium. After a two-hour period, the lobsters were removed from the labelled sea water, rinsed

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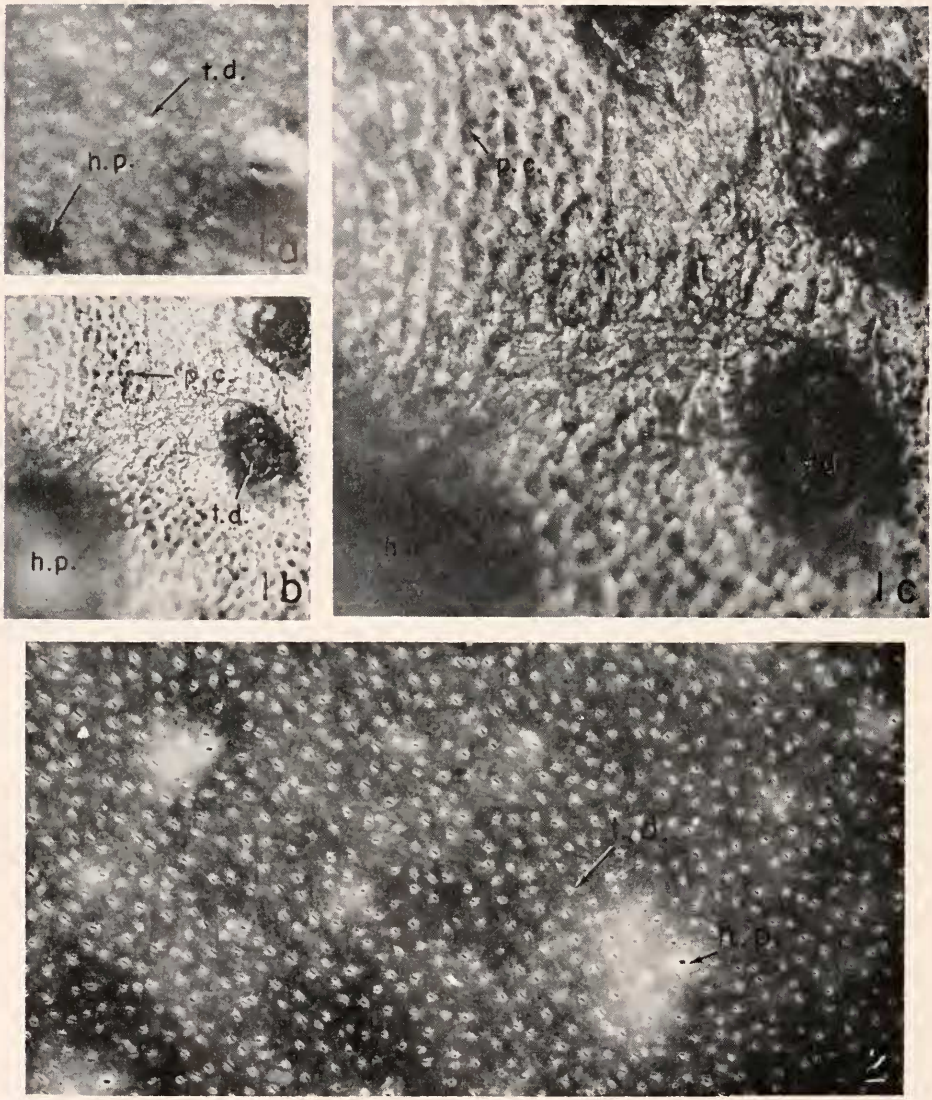


FIGURE 1. Surface view of dorsal claw shell, above muscle attachment.
FIGURE 1a. Obliquely reflected light. 30 × magnification.
FIGURE 1b. Directly reflected light. 250 × magnification.
FIGURE 1c. Directly reflected light. 500 × magnification.
FIGURE 2. Planar section, claw 1, principal calcified zone. Microroentgenogram. Enlarged 42 ×. h.p. = hair pore, t.d. = tegumental gland duct, p.c. = pore canal.

in a bath of distilled water, and blotted with gauze sponges. The posterior half of the carapace and the flat portion of the claw above the point of muscle attachment were removed. Pieces of shell about 20 mm.^{2} were obtained from the flat dorsal surface of the large member of the crusher claw above the point of muscle

attachment and 15 mm. from the tip of the claw. Carapace shell samples of similar size were also obtained at a point 15 mm. from the posterior and from the lateral borders of this tissue. The shells were rinsed briefly with a second jet of distilled water, and the non-calcified membrane was partially stripped off. Shells were dried at 100° C. for 12 hours.

A randomly selected area of dorsal claw shell, above the point of muscle attachment, was photographed at a magnification of 30 × by obliquely reflected light (Fig. 1a). By means of a metallurgical microscope, photographs of the shell by directly reflected light were obtained (Figs. 1 b-c). Dr. M. E. Nicholson of the Metallurgy Department of this University assisted with this procedure.

Irregular pieces of shell, about 16 $mm.^2$, were embedded in methyl methacrylate. One hundred-micron longitudinal and transverse sections were cut as well as one transverse 50-micron section from the carapace. The sections were polished on both sides with emery paper and placed directly upon the emulsion side of Kodak Maximum Resolution plates. Microroentgenograms were prepared according to the technique described by Bergman and Engfeldt (1954) and Engström *et al.* (1957). Target-to-film distance was at least 25 cm. Polychromatic radiation from a Machlett x-ray tube, with a tungsten target and a beryllium filter, with 6 KV tension, were used to obtain the microroentgenograms. The developed microroentgenograms were photographically enlarged. Positive prints of these enlargements are shown in Figures 2–8. In these figures, areas which are white in the microroentgenogram print represent areas of greater mineral concentration than areas with a gray cast.

To obtain photomicrographs of the claw sections, a reflected light source was used, while a transmitted light source was employed in the preparation of the photomicrographs of carapace sections.

Autoradiographs were prepared by placing the tissue sections in contact with the emulsion side of Kodak glass lantern slide plates. The tissues were allowed to remain in contact with the glass plates four months before the plates were developed. Darkening of the photographic film was shown to be due to beta particles from calcium-45 rather than gamma rays from strontium-85. Aliquots of solutions containing either calcium-45 or strontium-85 were pipetted onto blotter paper planchettes, designed so that a planchette of approximately the same size as a shell sample contained approximately the same amount of activity. After the planchettes were dry, they were placed in contact with the photographic plates for two months. No darkening due to strontium-85 gamma rays was observed when the plates were developed after this time.

The calcium content of adjacent fragments of shell was determined by the method of Clark and Collip (1925) after the shells had been ashed at 450–500° C. The calcium concentrations, expressed in mEq./g. dry shell, were as follows:

Carapace	1:	13.1 mEq./g.,	2:	13.8 mEq./g.
Claw	1:	11.5 mEq./g.,	2:	12.7 mEq./g.

The total residue remaining after ashing was as follows, expressed as per cent of dry weight:

Carapace	1:	67.7%	2:	70.1%
Claw	1:	60.5%	2:	66.6%

Description of photomicrographs, microroentgenograms, and radioautographs

Figure 1. Surface view of claw shell, randomly selected from dorsal surface of claw, above point of muscle attachment.

In Figure 1a, the openings of the hair pores (h.p.) and of the tegumental gland ducts (t.d.) are visible at 30 times magnification. In Figures 1b and 1c, 250 × and 500 × magnification, one hair pore is visible in the lower left hand corner. The tegumental gland duct openings (t.d.) are visible as large, circular structures. The many fine circular structures (p.c.) probably represent the points at which the pore canals initially opened to the surface. These figures were included to demonstrate the fact that there are three main types of openings or potential openings on the surface of the claw.

Figure 2 is a microroentgenogram, enlarged 42 times, of a planar section of the claw through the principal calcified zone (lobster 1). The microroentgenogram indicates that there are two circular or oval types of structure around which the density of the mineral is increased. Carlström (personal communication) has suggested that these could be areas in which the calcium carbonate possesses a crystalline structure. The larger dark spots (h.p.) surrounded by a bright halo probably represent transverse cuts through the pores of setae. They occur with about the same frequency as do the large pores identified as hair pores in Figure 1a. The smaller dark spots (t.d.) surrounded by areas of increased mineralization are the ducts of tegumental glands. The external openings of these ducts may be seen in Figures 1a-c. The slightly elliptical appearance of some of the passages in this microroentgenogram is due to the fact that the shell is curved and some of the pores have been cut at an angle greater or less than 90°.

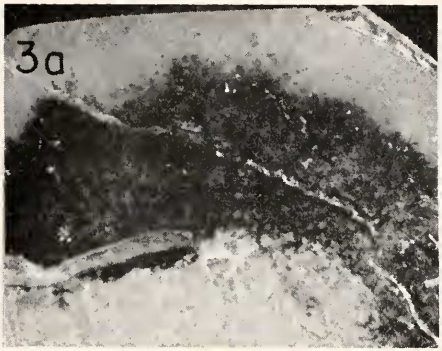
Figure 3a is a photomicrograph of a section which passes diagonally through the pigmented layer of the claw of lobster 2. The light triangular area on the right is methyl methacrylate as is the light area at the base of Figure 3a. The dark area in the center of the figure is the upper, heavily pigmented portion of the layer, and the lighter area immediately above represents the less heavily pigmented lower portion of this layer. The shell sample cracked when it was cut and polished and the break can be seen passing diagonally across the section.

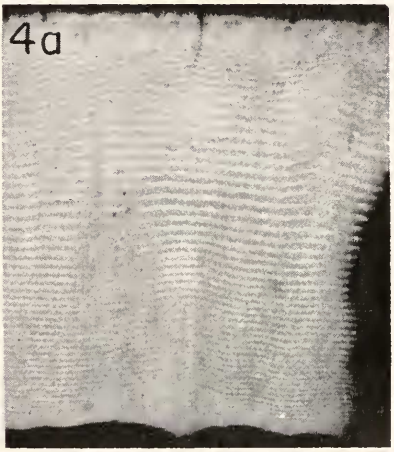
The microroentgenogram, Figure 3b, of this section of the claw indicates that there is slightly more mineralization in the outer strata than in the inner strata of this layer. Areas of lesser calcification probably represent ducts and pores or centers of cuticular prisms.

From the radioautograph of this section, Figure 3c, it is difficult to observe differential uptake of isotope in this layer. It appears that the lower and middle portions of the layer contain slightly more activity than the outermost portion.

Figure 4a represents the photomicrograph of a transverse section of claw (Lobster 1). The division between the pigmented layer and the principal calcified zone can be observed. There is some indication of the possible initial location of the interprismatic septa in the pigmented layer, although in *Homarus* the septa actually fuse at an early stage after the molt is completed (Yonge, 1932; Dennell, 1960).

In the microroentgenogram of this section of claw, Figure 4b, a thin layer of greater mineralization can be observed on the outer surface of the shell. This area probably corresponds to the region of the lower epicuticle and the upper pigmented layer. Drach (1939) stated that in the Brachyura, calcite crystals





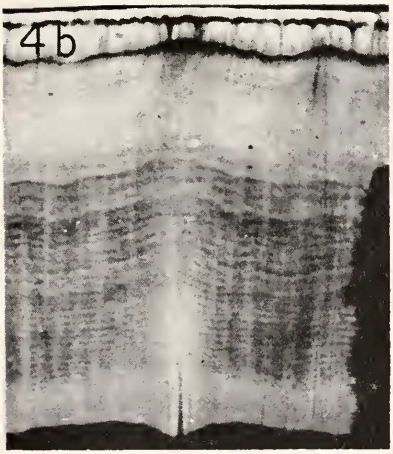


FIGURE 3. Planar section, claw 2, pigmented layer. Enlarged 48 ×.
FIGURE 3b. Microroentgenogram.
FIGURE 3c. Radioautograph.
FIGURE 4. Transverse section, claw 1. Enlarged 66 ×.
FIGURE 4a. Photomicrograph, reflected light.
FIGURE 4b. Microroentgenogram.
FIGURE 4c. Alternation of strips from microroentgenogram and radioautograph.

overlap between the two layers. The stratified nature of the pigmented layer, mentioned by Vitzou (1882), Herrick (1895), and Dennell (1960), is obscured by vertical "columns" of mineral material. The "columns" are wider at the outer surface. Dennell (1960) states that the interprismatic spaces are sites of early mineralization. From these sites, mineralization proceeds laterally and downward. It is possible that the areas which are less well mineralized represent interior portions of the cuticular prisms.

The strata of the principal calcified zone, underlying the pigmented layer, are clearly defined. The density of mineralization is actually less than that observed in the upper third of the pigmented layer. The most noticeable features of the principal calcified zone are the alternation in the density of the mineral matter in the horizontal strata and the two types of observed "ducts" which pass through the zone. The most numerous, clearly observed at the base of the principal zone, are probably the tegumental gland ducts. The large "duct" at the middle base of the principal zone is either an extremely large tegumental gland duct or the hair pore of a seta.

Figure 4c represents an attempt to correlate the strata of the principal calcified zone which may be observed in the microroentgenograms with those seen in the photomicrographs. Strips $\frac{1}{8}$ inch wide were cut, beginning at the same location, from both photomicrograph and microroentgenogram. The section was then reconstructed, using alternate strips from the two photographs. To some extent, the dark striations of the photomicrograph correspond to the dark striations of the microroentgenogram. The correlation is not perfect, and this can be regarded as suggestive only.

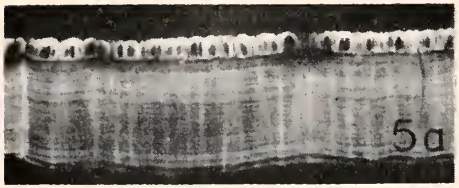
Figures 5a and 5b show the microroentgenogram and radioautograph obtained from transverse section from the claw of lobster 2. Details observed in 5a are similar to those seen in Figure 4b. There appears to be a greater concentration of the radioisotope in the outer layers of the claw.

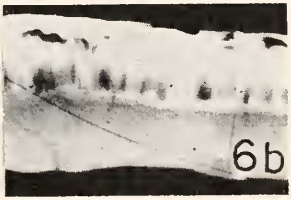
In Figure 6a, a transverse section of the carapace from lobster 2 is shown. This photomicrograph, obtained with transmitted light, merely indicates differences between outer and inner portions of the pigmented layer and the location of the principal calcified zone. The horizontal striations in the principal calcified zone, which can be easily observed at a broken edge of shell, are difficult to observe in this photograph. The very dark vertical line is an artifact.

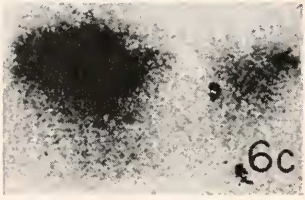
In the microroentgenogram of this section, Figure 6b, the mineral appears to be more uniformly distributed in the principal calcified zone. This may be due to greater density of the carapace shell as compared to the claw shell. In the radioautograph of the section, Figure 6c, calcium-45 activity is distributed in a much less uniform manner than in claw sections. There is little apparent relationship between uptake of isotope and variation in mineralization.

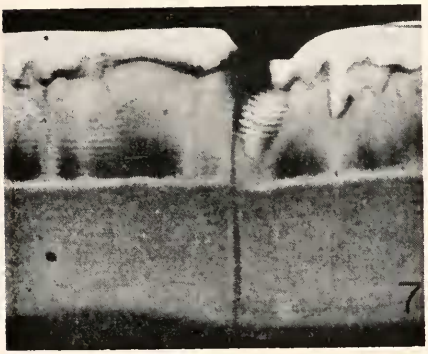
Figure 7 is a microroentgenogram of a carapace section, Lobster 1, half the thickness of that used to obtain the photographs in Figures 6a-b. There is more detail visible in the pigmented layer. However, strata in the principal calcified zone are only faintly visible, indicating that mineral is indeed more uniformly distributed in this zone in the carapace than in the claw. The duct observed is probably the hair pore of a seta.

Figures 8a, b, and c were obtained using a planar section of carapace (lobster 2)









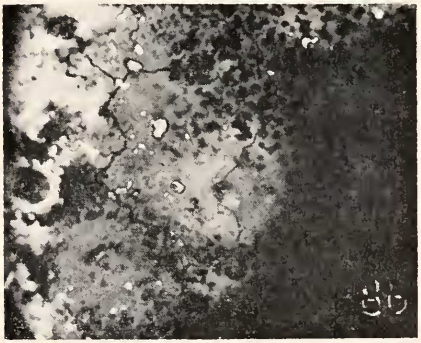


FIGURE 5. Transverse section, claw 2. Enlarged 22×.
FIGURE 5a. Microroentgenogram.
FIGURE 5b. Radioautograph.
FIGURE 6. Transverse section, carapace 2. Enlarged 66×.
FIGURE 6a. Photomicrograph, transmitted light.
FIGURE 6b. Microroentgenogram.
FIGURE 6c. Radioautograph.
FIGURE 7. Transverse section, carapace 1. Enlarged 98×. Microroentgenogram.
FIGURE 8. Planar section, carapace 2. Enlarged 60×.
FIGURE 8a. Photomicrograph, transmitted light.
FIGURE 8b. Microroentgenogram.
FIGURE 8c. Radioautograph. p.l. = pigmented layer; p.c.z. = principal calcified zone.

which passed through the pigmented layer and the principal calcified zone. In Figure 8a, the uniform gray area (p.c.z.) represents the principal calcified zone. The heavily pigmented, darker portion and the less heavily pigmented portion of the pigmented layer (p.l.) are visible.

In Figure 8b, the microroentgenogram of this section, the outermost portions of the pigmented layer appear to be heavily mineralized. The calcification suggesting interprismatic spaces is more clearly defined in the lower portion of the pigmented layer. In the principal calcified zone, the mineral is distributed in a uniform manner. The radioautograph, Figure 8c, shows again the irregular pattern of uptake of calcium-45 displayed by the carapace.

Discussion of photomicrographs, microroentgenograms, and radioautographs

Slight chemical differences have been observed among shell samples obtained from different areas of the exoskeleton of the lobster. The differences in density in these two areas apparent when Figures 4b and 5a are compared to Figure 6b could be a result of the slightly lower calcium concentration observed in the claw as compared to the carapace. The technique used to obtain the microroentgenograms is sufficiently sensitive to detect a difference of at least 15% in mineral density between two areas.

The irregular distribution of calcium-45 in the carapace, as compared to its distribution in the claw, suggests that the slightly denser mineral in the carapace contains a smaller percentage of labile calcium ions.

Drach (1939) pointed out that, in the integument of the crab, the area surrounding the hair pores may serve as a site for initial calcium deposits. The observation that some ducts and pores in the principal calcified zone are surrounded by denser mineral may bear some relation to Drach's finding.

The greater uptake of isotope by the pigmented layer, as compared to its uptake by the principal calcified zone, can be explained by the fact that the isotope was present in greater concentration at the outer surface of the shell.

General Summary and Conclusions

1. Visible light microscopy, microroentgenography, and radioautography were employed as techniques in the qualitative evaluation of the comparative distribution of mineral in the exoskeletons of lobsters and the relative exchangeability of calcium ions in the carapace and claw shells with the surrounding aqueous medium.

2. The following conclusions can be drawn from an examination of photographs obtained using these techniques: (1) In the claw and the carapace, a greater uptake of radiocalcium is observed in the outer portion of the shell. Radioactivity appears to be more uniformly distributed in the outer layer of the claw than in the same region in the carapace. (2) The outer third of the pigmented layer in the claw and the carapace contains the mineral material of greatest density. (3) In the claw, increased mineralization is observed in regions immediately surrounding the tegumental gland ducts and the hair pores; this increased mineralization is not observed in the carapace sections. The laminar nature of the principal calcified zone, observed in microroentgenograms of claw sections, is suggested only in carapace sections 50 microns in thickness. (4) The laminar nature of the pigmented layer of the carapace is obvious in microroentgenograms of cross-sections 50 microns in thickness.

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